AMENDMENTS

IN THE SPECIFICATION

1) On page 12, lines 31 through 32, please delete the paragraph and kindly replace it with the following new paragraph:

#1

Figure 4 illustrates rearrangements of 12q15 in human lipomas which disrupt the HMGI-C gene and produce chimeric transcripts (SEQ ID NOS: 1, 2, 3, 4, 5 and 6).

2) On page 13, lines 1 through 2, please delete the paragraph and kindly replace it with the following new paragraph:

#2

Figure 6(A) and 6(B) illustrate novel sequences fused to the DNA binding-domains of HMGI-C which encode transcriptional regulatory domains (SEQ ID NOS: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and 22).

3) On page 30, lines 13 through 35, please delete the paragraph and kindly replace it with the following new paragraph:

#3

To revert the obese phenotype, HMGI-C gene was inactivated by homologous recombination (Zhou et al., 1995) and the resulting mice were bred with the obese mutants. During the experiment, male and female mice were maintained under alternating 12-h light and dark periods and provided water and food ad libitum. Since both ob/ob and pg/pg animals are sterile (Green, 1989), crosses were carried out in two stages. First, a pg/ + Xob/ + intercross was undertaken and the progeny from this cross were genotyped using Southern blotting and PCR amplification. To screen for obese mutation, DNA was isolated from the mouse tails by standard methods (Sambrook et al., 1989) and PCR

amplified using sense primer 5'-CATTCTGAGTTTGTCCAAGATGC-3' (SEQ ID NO: 23) and antisense primer 5'-GGTCTGAGGCAGGGAGCAGC-3' (SEQ ID NO: 24). PCR conditions were as follows: denaturation at 95°C for 2 minutes and 30 cycles of amplification at 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 30 seconds, followed by a final extension for 10 minutes at 72°C. The resulting PCR products were digested with DdeI and electrophoresed on 8% polyacrylamide gel. Under these conditions, amplification of the wildtype allele yields 150 bp products which contains no DdeI restriction sites. The ob mutation substitutes T for C in position 369, generating a novel DdeI site. Therefore, digestion of the PCR product from mutant allele generates unique products of 106 and 44 bp. Genotyping of the HMGI-C knockout mice was carried out as described previously (Zhou et al., 1995). The double heterozygous animals (pg/+ ob/+) thus identified were intercrossed again and the double homozygotes (ob/ob pg/pg) obtained from this second cross were further analyzed.

4) Starting on page 40, line 32 and ending on page 41, line 5, please delete the paragraph and kindly replace it with the following new paragraph:

Initially, conserved fragments were isolated from the cloned, mouse pygmy locus (Xiang et al., 1990; K. Benson and K.C., unpublished observations) and were used as probes on a normal, human lambda genomic library (Sambrook et al., 1989). The cross-hybridizing clones were isolated and relevant homologous fragments were subcloned and sequenced. Specific oligonucleotide primers sequence 5'-AGGGGACAACAAATGCCCACAGG (SEQ ID NO: 25) and 5'-CGTCACCAGGGACAGTTTCACTTGG (SEQ ID NO: 26) were synthesized and used to screen a human total genomic YAC library by the PCR-based method (Green and Olson, 1990). Four positive clones of Saccharomyces cerevisiae containing YACs yWPR383, yWPR384, yWPR385 and yWPR386 were isolated.

5) On page 42, lines 6 through 29, please delete the paragraph and kindly replace it with the following new paragraph:

First strand cDNA was synthesized in a 20 ml reaction using an anchored oligo-dT primer 5'-GCAATACGACTCACTATAG$(T)_{13}$ (SEQ ID NO: 27) and Superscript II RT reverse transcriptase (BRL, Gaithersburg, MD) according to the manufacturer's protocol. Primers used in the first round of 3' RACE (Ausubel et al., 1989) were an HMGI-C exon 1 sense primer 5'-CTTCAGCCCAGGGACAACC (SEQ ID NO: 28) and an antisense adapter primer 5'- GCAATACGACTCACTATAG (SEQ ID NO: 29). One ml of first-strand cDNA was combined with 25 pmole of sense primer in a 50 ml reaction mixture (60 mM Tris-SO_4 (pH 9.1 at 25°C); 18 mM $(NH_4)_2SO_4$; 2 mM $MgSO_4$; each dNTP at 200 mM; 2.5 U of Taq DNA polymerase (BRL)), denatured for 2 minutes at 94°C and subjected to 5 cycles of linear amplification (Rother, 1992) using the following conditions: 94°C, 30 seconds; 58°C, 20 seconds; 72°C, 1 minute 30 seconds. Ten pmole of antisense primer were then added and 25 cycles of exponential amplification were performed (94°C, 30 seconds; 56°C, 30 seconds; 72°C, 1 minute 30 seconds). One ml of the PCR reaction was reamplified for 20 cycles with a nested HMGI-C sense primer spanning exon 1 and 2, 5'-GGAAGCAGCAGCAAGAACC (SEQ ID NO: 30) as described above. Five ml of each reaction were analyzed on a 1.5% agarose gel. Reverse transcription for the detection of chimeric transcripts using novel sequence-specific primers was performed as above except primers 375 (5'-CTTCTTTCTCTGCCGCATCG) (SEQ ID NO: 31) for ST90-375 and 724 (5'-GTGAGGATGATAGGCCTTCC) (SEQ ID NO: 32) for ST93-724 were used. Subsequent PCR conditions were an initial denaturation at 94°C for 2 minutes; 30 cycles at 94°C, 30 seconds; 58°C, 30 seconds; 72°C, 1 minute, followed by a final extension for 10 minutes at 72°C.

6) On page 43, lines 3 through 14, please delete the paragraph and kindly replace it with the following new paragraph:

H6

The NIGMS monochromosomal somatic cell hybrid mapping panel #2 was obtained from the Coriell Cell Repositories (Coriell Institute for Medical Research, Camden, NJ). Primers used were derived from the novel sequences of the chimeric transcripts and 500 ng of genomic DNA from each somatic cell line was used as a template for PCR amplification. For the novel sequence derived from the chimeric transcript obtained from lipoma ST90-375, the primers were 5'-CAGAAGCAGACCAGCAAACC (SEQ ID NO: 33) and 5'-CTTCTTTCTCTGCCGCATCG (SEQ ID NO: 34) and from lipoma ST93-724, the primers were 5'-CTCTGGAGCAGTGCAATGTG (SEQ ID NO: 35) and 5'-GTGAGGATGATAGGCCTTCC (SEQ ID NO: 36). PCR conditions for the ST93-724 novel sequence primers were 26 cycles of 94°C, 15 seconds; 64°C, 30 seconds; 72°C, 1 minute. For ST90-375, the same conditions were used except that the annealing temperature was 62.5°C. PCR products were analyzed on a 7% acrylamide gel.

7) On page 45, lines 23 through 32, please delete the paragraph and kindly replace it with the following new paragraph:

H7

Figure 4 illustrates rearrangements of 12q15 in human lipomas which disrupt the HMGI-C gene and produce chimeric transcripts (SEQ ID NOS: 1, 2, 3, 4, 5 and 6). HMGI-C denotes the nucleotide and amino acid sequence of the wildtype gene and the open box sequ3nce corresponds to the end of HMGI-C exon 3. t(3;12) (SEQ ID NOS: 3 and 4) and t(12;15) (SEQ ID NOS: 5 and 6) refer to the nucleotide and predicted amino acid sequences of the chimeric transcripts from the cloned cDNA products obtained by 3' RACE on RNA isolated from primary cell cultures of ST93-724, t(3;12) (SEQ ID NOS: 3 and 4), and ST90-375, t(12;15) (SEQ ID NOS: 5 and 6), respectively, Chr. 3 and Chr. 15

refer to the novel sequences derived from chromosome 3 or 15 in t(3;12) (SEQ ID NOS: 3 and 4) and t(12;15) (SEQ ID NOS: 5 and 6) lipomas, respectively. Only the sequences immediately adjacent to the fusion sites are shown.

8) On page 46, lines 11 through 28, please delete the paragraph and kindly replace it with the following new paragraph:

Figures 6(A) and 6(B) illustrate novel sequences fused to the DNA binding-domains of HMGI-C which encode transcriptional regulatory domains. Figure 6(A) illustrates a comparison of the novel chromosome 3 sequence from ST93-724 with the LIM domain-containing proteins, zyxin (Sadler et al., 1992), apterous (ap) (Cohen et al., 1992), Lh2 (Xu et al., 1993), Lin11 (Freyd et al., 1990), RBTN-1 (McGuire et al., 1989). Amino acids that constitute the LIM domain consensus are highlighted. The amino acid spacing between the consensus residues is indicated by an x. In addition to the totally conserved cysteine, histidine and aspartic acid residues (Sadler et al., 1992), LIM domains are characterized by the presence of an aromatic residue adjacent to the first histidine and a leucine located C-terminal to the central HxxCxxCxxC (SEQ ID NO: 20) cluster. The positions of these conserved residues are indicated by arrows. Each LIM domain is designated 1, 2 or 3 depending on its position relative to the N-terminus. The uninterrupted sequence of the two LIM domains in the various proteins are shown and gaps were introduced to permit alignment of the two LIM domains. Figure 6(B) illustrates the potential transactivation acidic domain encoded by the sequence derived from chromosome 15 in ST90-375. Acidic residues are underlined and the amino acids, serine and threonine, are in bold type.

9) Starting on page 47, line 28 and ending on page 48, line 24, please delete the paragraph and kindly replace it with the following new paragraph:

Methods. The 0.5kb *Apa*I-*Apa*I fragment (Xiang, X. et al., 1990) was used as a probe to isolate clones 3 and 4 from an EMBL3 mouse genomic library (a kind gift of Dr. E. Lacy) and a YAC 902CO711) from a mouse YAC library (Lehrach, H. et al., 1990). YAC 902CO711 was further subcloned into lambda FIX II (Ausubel, F. et al., 1988) and 86 clones that hybridized to radioactively-labeled mouse genomic DNA were picked and transferred to new plates in a gridded array (Ausubel, F. et al., 1988). Lambda clones, 802, 906, 5B, 803 and 308 were isolated after the walk was initiated with the 0.5kb *Apa*I-*Apa*I fragment and accomplished by repeated hybridization to filters of the array. Overlaps between the contig clones and colinearity with the genome were confirmed by a combination of clone to clone and clone to genomic hybridizations along with restriction mapping. Exon amplification was performed (Exon Trapping System, Gibco BRL) after the genomic inserts from the lambda clones were removed by cleavage with SalI, partially filled-in (Ausubel, F. et al., 1988) and subcloned into a partially filled-in BamH1 cleaved pSPL1 plasmid (Buckler, A. et al., 1991). The DNA was electroporated into COS-7 cells at 180V and 960mF in a Bio-Rad Gene Pulser. Cytoplasmic RNA was isolated after 2-3 days and RT-PCR performed using primers supplied by the manufacturer. The secondary PCR amplification products (Buckler, A. et al., 1991) from clones 803 and 5B were subcloned into the plasmid vector, pAMP10 (Exon Trapping System, Gibco BRL) and sequenced using the Sequenase Version 2.0 sequencing kit (USB) (Ausubel, F. et al., 1988). A 344bp fragment corresponding to the complete open reading frame of the HMGI-C gene (Manfioletti, G. et al., 1991) was amplified from 12.5dpc mouse embryos (see text) using reverse transcription (RT) and PCR. Lambda clones containing the HMGI-C exons were then isolated by hybridization of the 344bp radioactively-labeled fragment to the gridded array of lambda clones and subsequently connected through chromosome walking. The RT-PCR conditions for isolation of the 344bp fragment consisted of first strand cDNA synthesis with primer 1 (5'-ATGAATTCCTAATCCTCCTCTGC-3') (SEQ ID NO: 37) followed by PCR amplification with primers 1 and 2

#9 conclude

(5'-ATGGATCCATGAGCGCACGCGGT-3') (SEQ ID NO: 38). PCR conditions were 94°C, 0.5 minute; 55°C, 0.5 minute; 72°C, 1 minute; for 30 cycles. The amplified product was confirmed by sequencing analysis (Ausubel, F. et al., 1988).

10) Starting on page 51, line 30 and ending on page 53, line 8, please delete the paragraph and kindly replace it with the following new paragraph:

#10

The following 15- through 21-mer oligonucleotides are complementary to the human HMGI-C mRNA transcript beginning with the translation initiation codon:

5'-GCC CTC ACC GCG TGC GCT CAT-3', 3' (SEQ ID NO: 39)

5'-CC CTC ACC GCG TGC GCT CAT-3'3' (SEQ ID NO: 40)

5'-C CTC ACC GCG TGC GCT CAT-3'3' (SEQ ID NO: 41)

5'- CTC ACC GCG TGC GCT CAT-3'3' (SEQ ID NO: 42)

5'-TC ACC GCG TGC GCT CAT-3'3' (SEQ ID NO: 43)

5'-C ACC GCG TGC GCT CAT-3'3' (SEQ ID NO: 44)

5'- ACC GCG TGC GCT CAT-3'3' (SEQ ID NO: 45)

11) On page 52, lines 10 through 20, please delete the paragraph and kindly replace it with the following new paragraph:

#11

Similarly, the following 15- through 21-mer oligonucleotides are complementary to the human HMGI(Y) mRNA transcript beginning with the translation initiation codon:

5'-CTT CGA GCT CGA CTC ACT CAT-3' (SEQ ID NO: 46)

5'-TT CGA GCT CGA CTC ACT CAT-3' (SEQ ID NO: 47)

5'-T CGA GCT CGA CTC ACT CAT-3' (SEQ ID NO: 48)

5'-CGA GCT CGA CTC ACT CAT-3' (SEQ ID NO: 49)

#11 Conclude

5'-GA GCT CGA CTC ACT CAT-3' (SEQ ID NO: 50)

5'-A GCT CGA CTC ACT CAT-3' (SEQ ID NO: 51)

5'- GCT CGA CTC ACT CAT-3' (SEQ ID NO: 52)

12) On page 54, lines 18 through 28, please delete the paragraph and kindly replace it with the following new paragraph:

#12

Additional small molecule drugs which bind to HMGI proteins directly may be obtained by methods known to those skilled in the art. For example, HMGI protein or their fragments may be immobilized on scintillating plates and a library of various radiolabeled compounds can be screened against the plate using high-throughput screening equipment available commercially from, for example, Hewlett-Packard. Binding of a compound to an immobilized HMGI protein or its fragment will result in increased scintillation counts. Specific areas of HMGI proteins which present attractive targets are, for example, HMGI DNA-binding domains with a consensus sequence TPKRPRGRPKK (SEQ ID NO: 53) (Reeves and Nissen, 1990) or the sequence PRGRPKGSKNK (SEQ ID NO: 54) implicated in protein-protein interactions involving HMGI proteins (Leger et al., 1995).

Respectfully submitted,
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